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Cynthia Wright
Department of Chemistry, University of Alabama in Huntsville, Huntsville, Alabama 35899, USA

Bhuwan K. Chhetri
Department of Chemistry, University of Alabama in Huntsville, Huntsville, Alabama 35899, USA

William N. Setzer
Department of Chemistry, University of Alabama in Huntsville, Huntsville, Alabama 35899, USA

Correspondence:
William N. Setzer
Department of Chemistry, University of Alabama in Huntsville, Huntsville, Alabama 35899, USA
E-Mail: wsetzer@chemistry.uah.edu

Chemical composition and phytotoxicity of the essential oil of *Encelia farinosa* growing in the Sonoran Desert

Cynthia Wright, Bhuwan K. Chhetri, and William N. Setzer*

ABSTRACT

The essential oils from the leaves and stems of *Encelia farinosa* growing wild in Tucson, Arizona were obtained by hydrodistillation and analyzed by GC-MS. A total of 89 compounds were identified leaf and stem essential oils accounting for 95.7% of the leaf oil composition and 99.8% of the stem oil composition. The major components of the leaf oil were eupatoriochromene (20.8%), limonene (14.1%), α-pinene (11.6%), bicyclogermacrene (9.8%), sabinene (9.2%) and germacrene D (7.4%), while the stem oil was dominated by α-pinene (53.5%) and bicyclogermacrene (10.7%). The essential oils were screened for seed germination inhibition and seedling growth inhibition against *Lactuca sativa* (lettuce) and *Lolium perenne* (perennial ryegrass). Although the essential oils were relatively inactive in terms of inhibiting seed germination (about 40% germination inhibition of *L. sativa* and about 10% inhibition of *L. perenne* at 4.0 mg/mL), they both significantly inhibited radicle and hypocotyl elongation at 1.0 mg/mL

Keywords: *Encelia farinosa,* Essential Oil Composition, Allelopathy, A-Pinene, Sabinene, Limonene, Bicyclogermacrene, Eupatoriochromene.

1. Introduction

Encelia farinosa A. Gray ex Torr. (brittlebush), a member of the Asteraceae, is a perennial shrub that grows throughout the Sonora desert from Northern Mexico to Arizona. Its range includes the Mojave Desert and coastal chaparral of Southern California, and interior valleys of Southern California, and Southwestern Utah [1]. The plant reaches a height of two to five feet. It has fuzzy, grey-silver leaves and brittle, wooden stems. A beige gummy, sticky resin coats the wooden stems. This resin was utilized by the indigenous peoples to seal pottery, treat toothache [2], and as a chewing gum [3]. When cut, the branches and stems release fragrant, yellow oil that was used by early Catholic friars as incense. Brittlebush produces yellow flowers on long stalks in late winter and early spring.

Brittlebush has long been known for its phytotoxic effects [4]. 3-Acetyl-6-methoxybenzaldehyde has been extracted via ether from brittlebush and identified as a growth inhibitor toxic to tomato seedlings [5]. Two sesquiterpene lactones, farinosin and encelin (dehydro-farinosin), have also been isolated from brittlebush [6]. Chloroform extraction of brittlebush revealed farinosin to be the primary lactonic component of brittlebush leaves and encelin was indentified in the green stems of the plant [6]. The ethanol extract of brittlebush has yielded several substituted benzofurans and chromenes [7]. Although non-volatile phytotoxic compounds from *E. farinosa* have been identified, to our knowledge, neither the essential oil composition of this plant nor the phytotoxic effects of volatile components have been previously examined.

2. Materials and Methods

2.1 Plant Material

Plant material was obtained from Tucson, Arizona at Arizona-Sonora Desert Museum. Julie Wiens, of the Arizona-Sonora Desert Museum, identified and collected the *E. farinosa* samples (11.86 grams of stems and 9.98 grams of leaves). The materials were chopped and hydrodistilled using a Likens-Nickerson apparatus for four hours with continuous extraction of the distillates with chloroform gave yellow essential oils (575 mg and 348 mg from the stem and the leaves, respectively), which was stored at -20 °C until analysis.

2.2 Gas Chromatographic – Mass Spectral Analysis

The essential oils of *E. farinosa* were analyzed by GC-MS using an Agilent 6890 GC with Agilent 5973 mass selective detector [MSD, operated in the EI mode (electron energy = 70 eV), scan range = 40-400 amu, and scan rate = 3.99 scans/sec], and an Agilent ChemStation data system. The GC column was an HP-5ms fused silica capillary with a (5% phenyl)-polymethylsiloxane stationary phase, film thickness of 0.25 µm, a length of 30 m, and an internal diameter of 0.25 mm. The carrier gas was helium with a column head pressure of 48.7 kPa and a flow rate of 1.0 mL/min. Injector temperature was 200 °C and detector temperature was 280°C. The GC oven temperature program was used as follows: 40°C initial temperature, hold for 10 min; increased at 3°C/min to 200°C; increased 2°/min to 220 °C. A 1 % w/v solution of each sample in CH_2Cl_2 was prepared and 1 µL was injected using a 10:1 split ratio.

Identification of the oil components was based on their retention indices determined by reference to a homologous series of *n*-alkanes, and by comparison of their mass spectral fragmentation patterns with those reported in the literature [8] and stored on the MS library [NIST database (G1036A, revision D.01.00)/ChemStation data system (G1701CA, version C.00.01.080)]. The percentages of each component are reported as raw percentages based on total ion current without standardization. The essential oil compositions of the leaf and stem essential oils of *E. farinosa* are summarized in Table 1.

Table 1: Chemical compositions of *Encelia farinosa* leaf and stem essential oils

RI[a]	Compound	% Composition[b]		
		Leaves		Stems
852	(2*E*)-Hexenal	0.1±0.0		---
926	Tricyclene	---		0.1±0.0
935	α-Thujene	0.2±0.0		0.3±0.0
941	α-Pinene	**11.6±0.3**		**53.5±1.1**
954	Camphene	0.1±0.0		1.2±0.0
965	Benzaldehyde	tr[c]		---
976	Sabinene	**9.2±0.3**		0.8±0.0
978	β-Pinene	0.6±0.1		1.9±0.1
992	Myrcene	0.9±0.1		1.5±0.1
1004	α-Phellandrene	1.4±0.2		2.4±0.1
1016	α-Terpinene	tr		0.1±0.0
1024	*p*-Cymene	0.5±0.0		1.2±0.0
1028	Limonene	**14.1±0.6**		2.6±0.1
1038	(*Z*)-β-Ocimene	0.1±0.0		tr
1044	Phenylacetaldehyde	tr		---
1048	(*E*)-β-Ocimene	2.2±0.2		0.1±0.0
1058	γ-Terpinene	0.1±0.0		0.3±0.0
1088	Terpinolene	tr		0.2±0.0
1126	α-Campholenal	tr		0.1±0.0
1138	*trans*-Pinocarveol	---		0.1±0.0
1145	*trans*-Verbenol	tr		0.2±0.0
1161	Pinocarvone	tr		0.1±0.0
1165	Borneol	---		tr
1173	Umbellulone	0.1±0.0		---
1177	Terpinen-4-ol	---		0.1±0.0
1209	*iso*-Dihydrocarveol	---		tr
1215	Linalool formate	tr		---
1323	*neo*-Verbenol acetate	tr		---
1335	δ-Elemene	0.4±0.1		0.5±0.1
1339	7-*epi*-Silphiperfol-5-ene	---		0.1±0.0
1348	α-Cubebene	---		tr
1366	Cyclosativene	0.1±0.0		0.2±0.0
1372	Isoledene	---		0.3±0.0
1374	α-Copaene	0.1±0.0		0.7±0.0
1384	β-Bourbonene	0.1±0.0		tr
1390	β-Cubebene	0.1±0.0		---
1392	β-Elemene	0.1±0.0		0.2±0.1
1409	α-Gurjunene	---		0.7±0.0
1418	(*E*)-Caryophyllene	0.5±0.0		0.7±0.0

1427	β-Copaene	tr		0.3±0.0
1431	β-Gurjunene (= Calarene)	---		0.3±0.0
1433	γ-Elemene	tr		0.3±0.1
1436	α-*trans*-Bergamotene	tr		0.2±0.0
1438	Aromadendrene	tr		1.4±0.1
1439	α-Guaiene	0.1±0.0		---
1442	6,9-Guaiadiene	---		0.4±0.0
1453	α-Humulene	0.1±0.0		0.1±0.0
1458	(*E*)-β-Farnesene	0.1±0.0		---
1460	Alloaromadendrene	0.1±0.0		0.6±0.0
1472	9-*epi*-(*E*)-Caryophyllene	---		0.1±0.0
1477	*trans*-Cadina-1(6),4-diene	---		0.2±0.0
1482	Germacrene D	7.4±0.7		1.5±0.1
1485	γ-Selinene	---		0.4±0.0
1488	β-Selinene	---		0.3±0.0
1491	δ-Selinene	---		0.2±0.0
1495	Viridiflorene	---		1.0±0.1
1498	Bicyclogermacrene	**9.8±0.9**		**10.7±0.7**
1501	α-Muurolene	---		tr
1508	*trans*-β-Guaiene	---		0.2±0.1
1510	β-Bisabolene	0.7±0.1		2.3±0.2
1514	γ-Cadinene	---		0.4±0.1
1516	Cubebol	0.1±0.0		---
1524	δ-Cadinene	0.2±0.0		0.5±0.1
1550	Elemol	0.4±0.1		0.1±0.0
1556	Germacrene B	0.1±0.0		0.1±0.0
1567	(*E*)-Nerolidol	1.9±0.2		0.3±0.0
1576	Germacrene D-4-ol	0.3±0.0		---
1578	Spathulenol	2.8±0.2		1.9±0.1
1584	Caryophyllene oxide	1.1±0.1		0.8±0.0
1591	β-Copaene-4α-ol	0.1±0.0		---
1592	Viridiflorol	---		0.3±0.0
1594	Cubeban-11-ol	---		0.2±0.0
1594	Salvial-4(14)-en-1-one	0.1±0.0		---
1603	Rosifoliol	---		0.3±0.0
1608	*cis*-Isolongifolanone	0.6±0.1		---
1618	Unidentified[d]	3.0±0.2		---
1635	Isospathulenol	0.9±0.1		0.9±0.1
1642	τ-Cadinol	---		tr
1651	Desmethoxy encecalin	1.7±0.1		1.0±0.0
1655	Selin-11-en-4α-ol	1.0±0.1		---
1660	Ageratochromene	---		0.7±0.0
1680	Andro encecalinol	0.5±0.1		1.5±0.1
1684	α-Bisabolol	0.2±0.0		---
1686	Germacra-4(15),5,10(14)-trien-1α-ol	1.3±0.2		---
1689	(*Z*)-α-*trans*-Bergamotol	0.4±0.1		---
1701	(2*Z*,6*Z*)-Farnesol	tr		---
1739	Isobicyclogermacenal	0.1±0.0		---
1763	Eupatoriochromene	**20.8±0.2**		---
1813	2,2-Dimethyl-7-isobutyl-2*H*,5*H*-pyrano[4,3-b]-pyran-5-one	0.3±0.1		tr
1871	Encecalin	0.3±0.1		0.5±0.0
1891	Unidentified[e]	1.6±0.3		---
	Total Identified	95.7		99.8

[a] RI = "Retention Index" calculated in reference to a homologous series of *n*-alkanes on an HP-5ms column.
[b] The percentages of each component are averages ± standard deviations from three separate measurements and are reported as raw percentages based on total ion current without standardization.
[c] tr = "trace" (< 0.05%).
[d] MS (m/e): 206(18%), 162 (100%), 147(52%), 120(33%), 106(48%), 93(46%), 91(53%), 81(43%), 79(44%), 67(31%), 41(29%).
[e] MS (m/e): 216(79%), 201(100%), 173(16%), 115(14%), 91(7%), 69(7%), 43(7%).

2.3 Phytotoxicity Assays

An allelopathic bioassay based on lettuce (*Lactuca sativa*) and perennial rye grass (*Lolium perenne*) germination and subsequent radical and hypocotyls growth [9] was used to study the effects of the brittlebush oils. Stock solutions of each essential oil (4.0 g/L essential oil and 1.0g/L Tween-80 in water) were prepared and used for the assays. Two-fold serial dilutions of stock test solutions were prepared to give test concentrations of 4.0, 2.0, and 1.0 g/L with the control being 1.0 g/L aqueous Tween-80. Seeds were placed in 6-well test plates (10 seeds per well) each well lined with two layers of Whatman No. 1 filter paper moistened with test solution and the test plates were sealed with Parafilm®. The test plates were incubated at room temperature in the dark for 5 days, after which the number of germinated seeds was determined and the root (radical) and shoot (hypocotyl) lengths were measured. Student's *t*-test was used to compare radical and hypocotyls test means with controls [10].

3. Results and Discussion

3.1 Essential Oil Compositions

Hydrodistillation produced a yield of 0.48% and 3.49% oil for the stems and leaves, respectively. Sixty-eight compounds were identified in the stem oil and 64 were identified in the leaf oil. The chemical compositions of the oils are shown in Table 1. *E. farinosa* stem oil was dominated by α-pinene (53.5%) and bicyclogermacrene (10.7%). The leaf oil of *E. farinosa* was composed of eupatoriochromene (20.8%), limonene (14.1%), α-pinene (11.6%), bicyclogermacrene (9.8%), sabinene (9.2%), and germacrene D (7.4%).

3.2 Allelopathic Activity

The allelopathic potentials of *E. farinosa* oils have been assessed in terms of inhibition of seed germination and seedling growth against a representative dicot (lettuce, *Lactuca sativa*) and a representative monocot (perennial ryegrass, *Lolium perenne*). The allelopathic activities are summarized in Table 2. Both root (radical) and shoot (hypocotyls) growth of *L. sativa* and *L. perenne* were significantly inhibited by both the stem and leaf oils at concentrations as low as 1.0 mg/mL.

The allelopathic activity of the stem oil is probably not due to the abundant α-pinene concentration. At a concentration of 4.0 mg/mL, α-pinene has little effect on *L. sativa* or *L. perenne* seed germination, radicle or hypocotyl elongation [11]. The allelopathic activity of the stem oil must be due to other essential oil components, either alone or acting synergistically. The allelopathic activity of the leaf oil can be attributed to eupatoriochromene, which constitutes 20.8% of the leaf oil. Eupatoriochromene has been shown to reduce radical and hypocotyl growth [12]. The presence of limonene in the leaf oil may also contribute to the allelopathic activity of brittlebush. Limonene has been shown to inhibit the growth of both the radicle and hypocotyl of *Amaranthus viridis* [13] and *Triticum aestivum* [14], as well as *L. sativa* and *L. perenne* [9].

Table 2: Allelopathic activity of *Encelia farinosa* leaf and stem essential oils on lettuce (*Lactuca sativa*) and perennial ryegrass (*Lolium perenne*)

Concentration (mg/mL)	Germination Inhibition (%)		Seedling Growth (% of Controls)			
	Lactuca sativa	*Lolium perenne*	*Lactuca sativa*		*Lolium perenne*	
			radicle	hypocotyl	radicle	hypocotyl
Leaf EO						
4.0	40.0	28.3	65.1[a]	91.9[b]	72.0[a]	70.6[a]
2.0	36.7	11.7	80.8[a]	92.8[b]	78.0[b]	66.4[a]
1.0	43.3	10.0	89.9[b]	71.7[a]	77.9[b]	85.3[b]
Stem EO						
4.0	41.7	13.3	59.6[a]	57.4[a]	80.0[b]	33.5[a]
2.0	45.0	10.0	83.9[b]	85.8[a]	86.2[b]	67.8[a]
1.0	31.7	23.3	79.6[a]	89.8[b]	87.8[b]	70.3[b]

[a] Significantly different from control ($P<0.001$).
[b] Significantly different from control ($0.001<P<0.1$).

4. Conclusions

Volatile components in the stems and leaves of *Encelia farinosa* are likely to contribute to the observed allelopathic effects of this desert plant.

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